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DMRIE-C Reagent

Part no. 10459.pps MAN0000829 **Rev. Date** 14 July 2011

Cat. no. 10459-014 **Size** 1 mL **Store at 4°C (do not freeze)**

Description

DMRIE-C Reagent is suitable for transfecting DNA and RNA into eukaryotic cells, and is particularly effective for transfecting suspension cells (e.g. Jurkat) and other lymphoid-derived cell lines. Refer to the Cell Lines database at **www.invitrogen.com** for a list of cell types successfully transfected. DMRIE-C can also be used for *in vivo* delivery of DNA. DMRIE-C is a 1:1 (M/M) liposome formulation of the cationic lipid DMRIE (1,2-dimyristyloxy-propyl-3-dimethyl-hydroxy ethyl ammonium bromide) and cholesterol in membrane filtered water.

Important Guidelines for Transfection

- Form complexes using the recommended amounts of DNA (or RNA) and DMRIE-C. Optimize as necessary.
  Note: We recommend diluting DNA (or RNA) and DMRIE-C into Opti-MEM® I Reduced Serum Medium (Cat. no. 31985-062) before complexing.
- DMRIE-C is a lipid suspension that may settle with time. Mix thoroughly by inverting the tube 5–10 times before use.
- Transfect cells at the recommended confluence or cell density. Optimize as necessary. Maintain the same seeding conditions between experiments.
- *Do not* add antibiotics to media during transfection.
- For optimal results, perform transfection in medium without serum. Cells may be transfected in the presence of serum, if desired. However, complexes *must* be formed in serum-free medium.
- Test serum-free media for compatibility with DMRIE-C since some serum-free formulations (e.g. CD 293, 293 SFM II, VP-SFM) may inhibit cationic lipid-mediated transfection.

Intended Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Transfecting Adherent Mammalian Cells with DNA

Use the following procedure to transiently or stably transfect DNA into mammalian cells. All amounts and volumes are given on a *per-well basis*.

1. One day before transfection, plate cells in growth medium without antibiotics such that they will be at the recommended confluence at the time of transfection.

Condition	Cell no.	Growth med. vol.	Format	Confluence at time of transfection
Transient	$1–2 \times 10^5$	2 mL	6-well	60–80%
Stable	$1–2 \times 10^5$	4 mL	60-mm	30–50%

2. *For each transfection sample*, prepare complexes as follows:
   a. Dilute 1–2 µg of DNA in 500 µL (1 mL for stable transfection) of Opti-MEM® I Reduced Serum Medium (or other medium) without serum.
   b. Mix DMRIE-C before use, then dilute 2–12 µL of DMRIE-C in 500 µL (1 mL for stable transfection) of Opti-MEM® I Medium (or other medium) without serum. Let stand at room temperature for 30–45 minutes.
   c. Combine the diluted DNA with diluted DMRIE-C (total volume = 1 mL; 2 ml for stable transfection). Mix gently and incubate for 15–45 minutes at room temperature (the solution may appear cloudy).
3. Remove the growth medium from the cells and wash once with 2 mL of growth medium without serum. Remove the wash medium.
4. Add the complexes (from step 2c of this procedure) to cells. Mix gently by rocking the plate back and forth.
5. Incubate cells at 37°C in a CO_2 incubator for 4–24 hours.
6. Replace the medium with 2 mL of growth medium containing serum (4 mL for stable transfection). Alternatively, add 1 mL of growth medium containing 2X the normal concentration of serum (2 mL for stable transfection) without removing the DNA-containing medium.
7. **Transient:** Test for transgene expression 24–72 hours post-transfection.

   Stable cell lines: Passage cells at a 1:5 (or higher dilution) into selective medium 48–72 hours post-transfection.

Transfecting Suspension Mammalian Cells with DNA

Use the following procedure to transfect mammalian cells in suspension in a *6-well format*. All amounts and volumes are given on a *per-well basis.*

1. *For each transfection sample*, prepare complexes as follows:
   a. Aliquot 500 µL of Opti-MEM® I Reduced Serum Medium (or other medium) without serum into a well. Mix DMRIE-C before use, then dilute 2–12 µL into the medium, and mix gently by swirling the plate.
   b. In a 12 × 75 mm polystyrene tube, dilute 4 µg of DNA in 500 µL of Opti-MEM® I Medium (or other medium) without serum.
   c. Add the diluted DNA to the well containing diluted DMRIE-C (total volume = 1 mL). Mix by swirling the plate and incubate for 15–45 minutes at room temperature (solution may appear cloudy).
2. While complexes are forming, prepare a single-cell suspension from stock cells. Wash the cells once with serum-free growth medium without antibiotics, and add 2–3 × 10^6 cells in 0.2 mL of serum-free growth medium without antibiotics to each well.
3. Incubate cells at 37°C in a CO_2 incubator for 4–5 hours.
4. Add 2 mL of growth medium containing 15% fetal bovine serum to the cells. **Note:** For Jurkat and MOLT-4 cells, add 50 µg/mL PMA and 1 µg/mL PHA-L, if desired, to enhance promoter activity and gene expression. For K562 and KG-1 cells, add PMA alone.
5. Test for transgene expression 24–48 hours post-transfection.

Transfecting Mammalian Cells with RNA

Use the following procedure to transfect mammalian cells with RNA in a *6-well format*. All amounts and volumes are given on a per-well basis.

1. One day before transfection, plate 2–3 × 10^5 cells in 2 mL of growth medium without antibiotics such that they will be 80% confluent at the time of transfection.
2. Just before transfection, remove the growth medium from the cells and wash once with 2 mL of growth medium without serum. Remove wash medium.
3. *For each transfection sample*, prepare complexes as follows:
   To a 12 x 75 mm polystyrene tube, add 1 mL of Opti-MEM® I Medium (or other medium) without serum. Mix DMRIE-C, then dilute 2–12 µL into the medium. Mix or vortex briefly. Proceed to step 4 on page 4.

Transfecting Mammalian Cells with RNA, Continued

4. *For each transfection sample*, add 2–5 µg of RNA into the tube containing DMRIE-C and medium (from step 3 of this procedure, page 3). Mix or vortex briefly, then immediately add complexes to washed cells.
5. Incubate at 37°C in a CO_2 incubator for 4 hours, then replace transfection medium with complete growth medium.
6. Assay for expression of transfected RNA 16–24 hours post-transfection. **Note:** Capped and polyadenylated mRNA is translated more efficiently and is more stable within the cell.

Optimizing Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying cell density, DNA (or RNA) and DMRIE-C concentrations, and transfection incubation time.

Scaling Up or Down Transfections

Procedures are provided to transfect cells in a 6-well format (60-mm format for stable transfection with DNA). For other formats, vary the amounts of DNA (or RNA), DMRIE-C Reagent, cells, and medium used in proportion to the relative surface area of the tissue culture vessel.

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